REMARKS

Claims 16-25 are pending. No claim has been allowed. The amendments to the claims are to put the claims in form for allowance or appeal. No new search is believed to be necessary.

Claims 16 and 18 have been amended to define the mixed protein preparations in operational terms which is supported by the Specification on page 7. The applicant defines "intracellular proteins" as the proteins removed from disrupted cells separated from the medium used to grow the cells. The amendment defines the intracellular protein preparation by the manner in which it is prepared, which is supported in the Specification on page 7, lines 3-15. The Applicant defines the term "extracellular proteins" as the proteins removed from the medium used to grow the cells. The amendment defines the extracellular protein preparation by the manner it is prepared, which is supported in the Specification on page 7, lines 3-7. Both Claims 16 and 18 were further amended to limit the claims to the vaccine disclosed in Example 1, which does not contain material less than 10,000 MW. Claims 19, 24, and 25 have been amended to conform to the amendments made to Claim 18 and to further limit the claims to the vaccine disclosed in Example 1.

The amendments are for purposes of putting the

claims in proper form for allowance or appeal. The amendments narrow the claims to the method enabled by the examples in the specification. Therefore, it is believed that the amendments would not require any further search by the Examiner.

The Applicant understands that the Examiner is asserting in the Office Action that every protein is an intracellular protein regardless of whether the protein functions as an intracellular protein or an extracellular protein because every protein is made in the cytoplasm of a cell. Therefore, an extracellular protein is both an intracellular protein and an extracellular protein. Applicant further understands that the Examiner is arguing that because newly synthesized proteins destined to be extracellular proteins are present in the cell from the time they are fully synthesized to the time they are extruded, any preparation of intracellular proteins will always contain at least one extracellular protein, which means any intracellular protein preparation is a mixture of intracellular and extracellular proteins. Thus, making it impossible to make a preparation consisting essentially of either extracellular proteins or intracellular proteins. The Applicant disagrees with the Examiner's argument as it applies to Applicant's vaccine for the following reasons.

The terms "extracellular" and "intracellular" are operational terms of art. The term "intracellular protein" refers to a protein destined to serve its life inside the cell and the term "intracellular protein preparation" refers to a preparation of proteins isolated from a disrupted cell after the media surrounding the cell has been removed. Any newly synthesized protein in an intracellular protein preparation that is destined to become an extracellular protein is, in most cases, present in a negligible amount and is, in most cases, not properly processed to be an extracellular protein. It is more accurate to refer to such proteins as precursors to extracellular proteins and not extracellular proteins.

The term "extracellular protein" refers to a protein destined to serve its life in the extracellular environment and the term "extracellular protein preparation" refers to a preparation of proteins that have been isolated from the media surrounding a cell. In general, extracellular proteins are distinguishable from their intracellular precursors because of the processing (e.g., cleavage, phosphorylation, glycosylation) such proteins undergo before being excreted into the extracellular environment.

One with ordinary skill in the art is fully aware of the general meaning of the terms

"intracellular" and "extracellular". When one of ordinary skill in the art is told that a protein preparation consists of extracellular proteins, the ordinary artisan understands that preparation consists essentially of those proteins excreted from cells, and when the ordinary artisan is told that a preparation consists of intracellular proteins, the ordinary artisan understands that the preparation consists essentially of those proteins that are ordinarily found only inside the cell.

In the instant application, the intracellular protein preparation consists essentially of proteins that are found only in the cell. Any contribution to the intracellular protein preparation by any newly synthesized precursor protein destined to be extracellular is expected to be negligible and its effect on the biological properties of the intracellular preparation is expected to be de minimis.

In the instant application, the extracellular protein preparation consists essentially of proteins that have been excreted from the cell. The fact that the extracellular proteins had been made in the cell is irrelevant because the extracellular protein preparation excludes all proteins that are within the cell.

While the Applicant disagrees with the Examiner's interpretation of the terms "intracellular"

and "extracellular" as they apply to Applicant's vaccine, to make clear what it is the Applicant is claiming, the Applicant has amended Claims 16 and 18 to define the intracellular and extracellular protein preparations by the manner they are prepared. Claims 19 and 24 were amended to be consistent with the amendments to Claim 18.

1. Claim 21 was rejected under 35 U.S.C. § 112, first paragraph, because the Specification lacks complete deposit information for ATCC 74446.

The paragraph beginning at page 5, line 19 was replaced with a new paragraph that adds the date of deposit to line 21 and corrects "ATCC 58643" on line 23 to "ATCC 74446." In view of the Applicant's Supplemental Declaration Under 37 C.F.R. 1.132 (filed December 4, 2000), which declares in paragraph (5) that ATCC 74446 and ATCC 586643 are the same, the ATCC catalog entries attached to the Appeal Brief submitted April 27, 2000, and the Declaration of Biological Deposit filed October 8, 1999, it is believed that this amendment does not introduce new matter into the application.

Claim 21 is now believed to be fully supported by the Specification. Reconsideration of the rejection is requested.

2. Claims 16-25 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Mendoza *et al.* (1996), Mendoza *et al.* (1992a) (IDS: AI), Mendoza *et al.* (1992b) (IDS: AJ), Panella *et al.* (1990), Sigma Catalogue (1992), and Amicon Catalogue (1993).

The Applicant believes that the prior art does not render the instant invention obvious, particularly in view of the claims as amended herein for the following reasons.

Mendoza (1992a) teaches two distinguishable vaccines. The first vaccine is a cell-mass vaccine (CMV) that the contains a mixture of proteins removed from cells and cell debris. The CMV does not contain proteins excreted into the medium. In contrast, the Applicant's intracellular protein component of the instant vaccine consists solely of those proteins removed from the disrupted cells. The intracellular component does not contain proteins excreted in the medium and it does not contain cell debris (Specification: page 7, lines 8-15).

The second vaccine taught by Mendoza (1992a) is a soluble concentrated antigen vaccine (SCAV) that contains solely the proteins excreted into the medium, concentrated in a stir cell, acetone precipitated, and resuspended in saline. The Applicant's extracellular protein component of the instant vaccine contains solely

proteins excreted into the medium that have not been concentrated in a stir cell. Instead, the extracellular protein component is mixed with the intracellular component, precipitated with acetone, resuspended in water, and dialyzed against water to remove material less than 10,000 MW.

In particular, Mendoza (1992b) teaches an intracellular protein preparation that does not contain the disrupted cell debris; however, the preparation is not in water and contains material that may not be precipitated by acetone and material that is less than 10,000 MW. Mendoza (1992b) teaches using the preparation in SDS polyacrylamide gel electrophoresis to identify immunodominant proteins in the preparation such as the 28, 30, and 32 kD proteins. Mendoza (1992b) teaches that the 28, 30, and 32 kD proteins may be useful for diagnostic purposes and immunotherapy.

Mendoza (1992b) further teaches removing the cell debris from the intracellular protein preparation by centrifugation. However, Mendoza (1992b) does not teach precipitating the proteins in acetone to remove material that cannot be precipitated with acetone and concentrate the proteins, resuspending the precipitated proteins in water, and then dialyzing the resuspended proteins in water to remove material less than 10,000 MW.

Mendoza (1992b) teaches using a PM10 membrane in a stir cell to concentrate extracellular proteins of *Conidiobolus coronatus* (Mendoza (1992b): page 2981) and Mendoza (1992a) teaches using a stir cell to concentrate the extracellular proteins of *Pythium insidiosum*. Neither teaches using a stir cell to concentrate intracellular proteins. In both Mendoza (1992a) and Mendoza (1992b), the proteins were concentrated under positive pressure, which reduces the volume of the filtrate by forcing liquid containing the proteins through the membrane. However, the stir cell does not remove the medium and replace it with water nor does it remove material less than 10,000 MW. The PM10 membrane merely prevents material greater than 10,000 MW from being lost as the volume of the filtrate is reduced during the process of forcing the filtrate through the membrane. Therefore, while the concentration of material greater than 10,000 MW in the filtrate increases as the volume of the filtrate is decreased, the concentration of material in the filtrate less than 10,000 MW and the solvent remains the same. Thus, after concentrating the filtrate using a stir-cell, the filtrate still contains the same concentration of less than 10,000 MW material and the same solvent.

In contrast to concentrating a sample using a stir-cell, dialysis works under the principle of

equilibrium to remove small molecules and exchange solvents. In dialysis, a sample such as the above filtrate is placed in bag consisting of a dialysis membrane, which is then placed in a large volume of a solvent. The membrane allows small molecules to pass freely through the membrane while retaining larger molecules which cannot pass through the dialysis membrane. Dialysis membranes are available with different MW cut-offs. For example, the dialysis membrane used by the applicant allows only molecules less than 10,000 MW to pass through the dialysis membrane. Therefore, when the sample in the dialysis membrane is placed in a large volume of solvent that does not contain the small molecules in the sample, the small molecules diffuse from the sample into the solvent until equilibrium is reached wherein the concentration of small molecules in the solvent becomes the same as the concentration of small molecules in the sample. Thus, the concentration of small molecules in the sample is reduced relative to the concentration of large molecules. By changing the solvent each time after equilibrium is reached, the concentration of small molecules in the sample can be completely removed or reduced to a negligible level. The same principle enables the solvent of the sample to be exchanged with another solvent.

Except in the case where the large volume of solvent contains a high salt concentration, dialysis does not result in the sample becoming concentrated; dialysis merely changes the composition of the sample by removing or introducing small molecules that can pass through the membrane. Therefore, a protein preparation containing low molecular weight material that is dialyzed results in a preparation that is distinguishable from the product that results when the same protein preparation is concentrated using a stir-cell because in the former the concentration of low molecular weight molecules is reduced or removed whereas in the latter the concentration of low molecular weight material remains the same.

Even if one skilled in the art were motivated to combine both the extracellular proteins prepared as taught in Mendoza (1992a) and the intracellular proteins as prepared as taught in Mendoza (1992a or 1992b), the resulting preparation would not have been the same as the protein preparation taught by the Applicant. The concentrated sample would still contain material less than 10,000 MW because the stir-cell concentrator merely reduces the volume of a sample; it does not cause the sample to no longer contain material less than 10,000 MW. There is nothing in Mendoza (1992b) and Mendoza (1992a) which would have made it obvious to precipitate

the proteins with acetone to remove material that cannot be precipitated by acetone and concentrate the proteins, resuspend the precipitated proteins in water, and dialyze the resuspended proteins in water to remove all the material less than 10,000 MW. Thus, merely combining the intracellular protein preparation for SDS polyacrylamide gel electrophoresis taught in Mendoza (1992b) with the extracellular protein-containing SCAV taught in Mendoza (1992a) would not have produced the Applicant's vaccine.

Furthermore, Mendoza (1992a) teaches that the SCAV is preferred over the CMV vaccine (Mendoza (1992a): abstract). Thus, Mendoza (1992a) teaches away from a vaccine that contains intracellular proteins. This also support's the Applicant's contention that the Applicant's vaccine is not obvious in light of Mendoza (1992a) and Mendoza (1992b). Furthermore, the Applicant's vaccine was shown to have the unexpected property of being able to cure horses that are chronically infected with *Pythium insidiosum* (Specification: page 8, lines 22-27), in addition to being able to cure horses that are acutely infected (Specification: page 8, lines 32-33). Because the prior art intracellular and extracellular vaccines cannot cure chronically infected horses whereas that the Applicant's vaccine can, it is clear that the admixed proteins

prepared as taught by the Applicant are acting in a synergistic manner to cure chronically infected horses. In contrast to the Applicant's vaccine, the results in Mendoza (1992a) showed that the prior art vaccines cured 0% of the chronically infected horses. The improved cure rate of chronically infected horses effected by the Applicant's vaccine is a significant improvement over the 0% cure rate of the prior art vaccine. Therefore, not only is the Applicant's vaccine a new composition that is not anticipated by the prior art, it also has the ability to cure chronically infected horses, a property that would not have been obvious to one skilled in the art in view of Mendoza (1992a) and Mendoza (1992b).

It was further remarkable and unobvious that the Applicant's vaccine prepared could cure a boy chronically infected with *Pythium insidiosum* (Specification: page 9, Example 4). Humans and horses are evolutionarily very distinct and it is well known that veterinary treatments and human treatments are not generally interchangeable. Therefore, it would not have been obvious that a veterinary vaccine for curing horses infected with *Pythium insidiosum* would have been able to cure a boy infected with *Pythium insidiosum*.

Amicon and Sigma in view of Mendoza (1992a) and Mendoza (1992b) still would not render the

Applicant's vaccine obvious. The copy of the two pages from the Sigma catalogue listing Thimerosal and the trademark for thimerosal, Merthiolate®, for sale with no description of the compound other than its chemical name fails to have any relevance to this rejection. The copy of the page from the Amicon catalogue indicating that Amicon sells PM10 membranes for stir cells is also of no particular relevance to the rejection on hand. In view of the prior art, the Sigma and Amicon references do no more than tell one of ordinary skill in the art where to buy thimerosal and P10 membranes.

Panella in view of Mendoza (1992a), Mendoza (1992b), Amicon, and Sigma still would not render the Applicant's vaccine obvious. The Examiner states that Panella teaches that thimerosal is a preservative that should be removed to eliminate non-specific immune responses. Panella does not appear to discuss the effects of thimerosal on the immune system. Instead, Panella discloses that thimerosal induces terminal differentiation of leukemic blasts and three human leukemic cell lines, and accelerated differentiation in normal bone marrow erythroid tissue. While one with ordinary skill in the art may use thimerosal to kill the cells as suggested in Panella, combining that disclosure with Mendoza (1992a) and Mendoza (1992b) would not have led to the Applicant's vaccine for the reasons discussed

with respect to Mendoza (1992a) and Mendoza (1992b).

Mendoza (1996) in view of Mendoza (1992a), Mendoza (1992b), Amicon, Sigma, and Panella still would not render the Applicant's vaccine obvious. Mendoza (1996) discloses "that the addition of cytoplasmic antigens, containing the 28K, 30K and 32K immunodominant proteins, to the original Pythium-vaccine enhanced its curative properties . . ." (Mendoza (1996): p 159). This statement is unclear as to whether the enhanced vaccine contains intracellular antigens including the three immunodominant proteins or the three immunodominant proteins separated from the other intracellular proteins. The title of the section this statement appears, "Western Blot (WB)," and the abstract (filed January 21, 2000) cited as the source for the statement suggest that the enhanced vaccine consists of culture filtrate antigens (CFA) mixed with the three immunodominant proteins separated from other intracellular proteins. The statement does not clearly suggest a vaccine like the Applicant's vaccine.

Therefore, that statement does not render the Applicant's vaccine obvious because even if one with ordinary skill in the art combined the intracellular proteins made according to Mendoza (1992b) with the extracellular proteins made according to Mendoza (1992a) in an attempt to achieve the enhanced vaccine alluded to

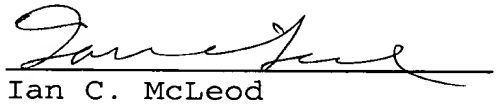
in Mendoza (1996), the resulting vaccine would contain some material less than 10,000 MW and would contain some intracellular material that is not acetone precipitable. Such a vaccine does not have the same composition as the Applicant's vaccine and, therefore, would not be expected to have the same remarkable properties as the Applicant's vaccine. Mendoza (1996), like the other prior art references, does not suggest to one of ordinary skill in the art the particular process that was used to make Applicant's vaccine.

Therefore, for the above reasons, the Applicant's vaccine is not rendered obvious in view of the prior art. Reconsideration of the rejection is requested.

Claims 16, 18, 19, and 24 have been amended to place the claims in proper form for allowance or appeal. Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The

attachment is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**" Therefore, entry of this amendment for purposes of allowance or appeal is requested.

Respectfully,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning at page 5, line 19 has been amended as follows.

The *Pythium insidiosum* strain was deposited with the American Type Culture Collection under the Budapest Treaty as ATCC 7446 on June 1, 1998. It is available upon request by name and number. All restrictions on distribution of ATCC [58643] 7446 are irrevocably removed on granting of a patent on this application. The address of the American Type Culture Collection is 10801 University Boulevard, Manassas, Virginia 20110-2209.

In the Claims:

Claims 16, 18, 19, 24 and 25 have been amended as follows.

A method for treatment of Pythiosis in human patients having the disease which comprises:

(a) providing a vaccine containing a mixture of proteins of *Pythium insidiosum* in a sterile aqueous solution, wherein the mixture of proteins [is] consists of (1) [of] mixed [intracellular] proteins removed from disrupted cells of the *Pythium insidiosum* [grown in] separated from a culture medium used to grow the cells and (2) [of] mixed [extracellular] proteins removed from the culture medium [for growing the] separated from the *Pythium insidiosum* wherein the [mixed intracellular proteins and the mixed extracellular proteins in water have] mixture in water has been dialyzed to remove low molecular weight components less than 10,000 MW; and

(b) vaccinating the patient with the vaccine.

A method for the treatment of Pythiosis in a mammal having the disease which comprises:

(a) providing an injectable vaccine derived from growing cells of *Pythium insidiosum* in a culture medium which comprises in a sterile aqueous solution in admixture:

(1) mixed [intracellular] proteins removed from disrupted cells of the *Pythium insidiosum* separated from the culture medium; and

(2) mixed [extracellular] proteins removed from [a supernatant from growing] the culture medium separated from the cells of the *Pythium insidiosum*;

wherein the [mixed intracellular proteins and the mixed extracellular proteins in water have] admixture in water has been dialyzed to remove low molecular weight components less than 10,000 MW to [product] produce the vaccine; and

(b) vaccinating the mammal with the vaccine.

The method of Claim 18 wherein the removed proteins in the admixture have been provided by growing cells of the *Pythium insidiosum* in the culture medium, then killing the cells, then separating the killed cells from the culture medium to produce a first supernatant containing the mixed [extracellular] proteins of step (2) and then disrupting the killed cells in sterile water and removing the disrupted cells to provide the mixed [intracellular] proteins of step (1) in a second supernatant [and separating the mixed intracellular proteins from the disrupted cells and removing the mixed extracellular proteins from the first and second supernatants], combining the first and second supernatants, precipitating the proteins, resuspending the precipitated proteins in sterile water, and dialyzing the resuspended proteins in sterile water to remove the material less than 10,000 MW.

-24-(Third Amended)

The method of Claim 19 wherein the [mixed intracellular protein in the second supernatant] disrupted cells are removed from the culture medium for the cells [are separated from the disrupted cells] by centrifugation [and removal of the disrupted cells] to provide the mixed proteins of step (1) in the second supernatant.

-25-(Third amended)

The method of Claim 19 wherein the mixed [intracellular and extracellular] proteins from steps (1) and (2) [have been] are precipitated [together from a combined mixture of the first and second supernatants] using acetone [and then isolated and dispensed in sterile distilled water to provide the vaccine].